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Letter to the Editor: Backbone ^{1}H, ^{15}N and ^{13}C resonance assignments of the *Staphylococcus aureus* acyl carrier protein synthase (AcpS)

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Biological context

The occurrence of resistant bacterial strains has made conventional antibiotics less effective (Hand, 2000). Therefore, the discovery of novel classes of anti-microbial agents has become important. Fatty acid biosynthesis, which is an essential biochemical pathway in all cellular organisms, has emerged as a promising target for the development of novel therapeutic agents. A central component of fatty acid biosynthesis is the small acidic protein, acyl carrier protein (ACP), which is required during all stages of synthesis. ACP exists in both an active (holo) and inactive (apo) form in which activation of ACP is mediated by holo-acyl carrier protein synthase (AcpS). AcpS catalyzes the transfer of the 4′-phosphopantetheinyl moiety from coenzyme A (CoA) to a conserved serine residue of apo-ACP to yield holo-ACP and 3′,5′-ADP in a Mg^{2+}-dependent reaction (Elovson and Vagelos, 1968). The ACP and AcpS proteins are both conserved across gram positive and gram negative bacteria making these targets suitable for identifying broad-spectrum antibacterial compounds. In contrast, neither protein exhibits significant homology to known eukaryotic proteins suggesting that bacterial specific inhibitors could be identified. The AcpS enzyme is a small protein of ∼14 kDa and was first identified in *E. coli* (Lambalot and Walsh, 1995). Homologues of AcpS have been identified in many bacteria with available genome sequences. X-ray crystal structuresof AcpS from *Bacillus subtilis* (Parris et al., 2000) and from *Streptococcus pneumoniae* (Chirgadze et al., 2000) revealed an α/β fold and showed that the enzyme assembles as a tightly packed homotrimer containing three active sites at the interface between protomers. The structures of *Bacillus subtilis* AcpS in complex with CoA and ACP have also been determined (Parris et al., 2000). The CoA binding site is located within the interface of two AcpS molecules in the homotrimer, while ACP interacts mainly with a positively charged molecular surface of AcpS via charge-charge interactions. As part of our efforts to use NMR techniques to assist in deciphering the enzyme's structure-function relationships and developing novel antibiotics, we report here the sequence-specific backbone resonance assignments of *Staphylococcus aureus* (S. aureus) AcpS. *S. aureus* AcpS has 49% and 37% sequence identity to *Bacillus subtilis* AcpS (1F7T.pdb) and *Streptococcus pneumoniae* AcpS (1FTH.pdb), respectively.

Methods and results

S. aureus AcpS was produced using a published protocol (Lambalot and Walsh, 1997) with modifications. Recombinant *S. aureus* AcpS was overexpressed in *E. coli* strain BL21(DE3). The cell cultures were grown at 37 °C in M9 minimal medium with $^{15}NH_4Cl$ as the sole nitrogen source and/or ^{13}C-glucose as the sole carbon source. 99% D_2O was used in M9 medium to produce perdeuterated protein samples. Recombinant protein expression was induced with IPTG (1 mM final concentration) at an optical density (600 nm) of 0.7. The protein yield was typically around 15–20 mg l^{-1}. Samples containing 0.3–1.5 mM protein

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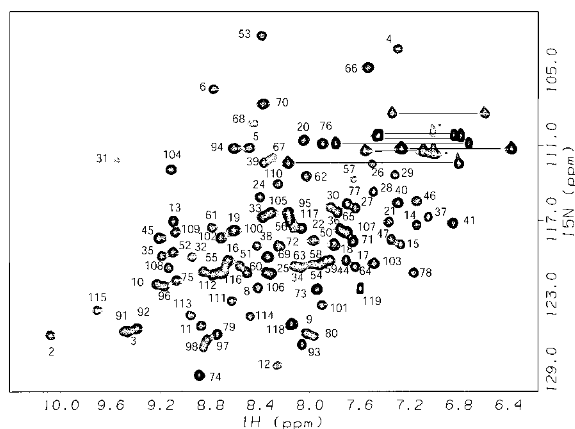

Figure 1. Two dimensional ^{1}H-^{15}N HSQC of AcpS acquired at 600 MHz in a buffer system containing 75 mM K_iPO_4, pH 7.5, 5 mM DTT, 0.015% NaN_3, 5% D_2O. The cross peaks in the spectrum are labeled with the assigned amino acid residue number. Pairs of peaks connected by a horizontal line are from NH_2 resonances of Asn and Gln side chains. Peaks labeled with an asterisk are from arginine side chains.

were prepared in 75 mM K_iPO_4, pH 7.5, 5 mM (DTT), 0.015% NaN_3, 0.4 mM 4-(2-Aminoethyl)-benzenesulfonyl fluoride (AEBSF), 5% D_2O.

NMR experiments were performed at 25 °C on a Varian *INOVA* 600 MHz spectrometer. Spectra were processed with FELIX980 (Accelrys Inc.) and analyzed with NMRView (Johnson and Blevins, 1994) on a Silicon Graphics workstation. Triple resonance experiments (Proteinpack, Varian Inc.), such as HNCA, HN(CO)CA, CBCA(CO)NH, CBCANH, HNCO, TROSY-HNCACB, together with a ^{15}N-edited NOESY-HSQC, acquired using a single ^{2}H-^{13}C-^{15}N triple labeled sample, were used to obtain ^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ and ^{13}C′ resonance assignments.

Extent of assignments and data deposition

The native *S. aureus* AcpS contains 119 amino acids with a molecular weight of 13.6 kDa per monomer. The ^{15}N-^{1}H HSQC spectrum of *S. aureus* AcpS is shown in Figure 1. The single set of cross peaks and their broad linewidths in the HSQC spectrum together with data from size exclusion chromatography are consistent with formation of a homotrimer in solution which was observed in the crystal structures of AcpS (Parris et al., 2000; Chirgadze et al., 2000). Due to the large molecular weight of the homotrimer (∼ 41 kDa) perdeuteration was necessary to obtain good quality triple resonance and ^{15}N-edited NOESY-HSQC spectra. Amino acid type-specific ^{15}N-labeled protein samples were prepared to assist in the identification of amino acid types. This was necessary for residues lacking ^{13}Cβ chemical shifts due to missing HNCACB peaks.

Of the possible 117 non-proline backbone amide resonances, 12 were not observed in a 2D ^{1}H-^{15}N HSQC spectrum and therefore, were not assigned (residues 1, 42, 49, 81–89) (Figure 1). Missing peaks are presumed to be from residues in slow conformational exchange and consequently broadened beyond detection. Most unassigned residues appear to be clustered within a short β hairpin region (81–89) which forms part of CoA binding site. ^{13}Cα, ^{13}Cβ, and ^{13}CO resonance assignments were obtained for 108, 100, and 104 residues, respectively. The ^{1}H, ^{15}N, and ^{13}C chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5493.

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